

## Phytoprotection



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J.-C. Côté, C. Vincent, K.-H. Son et S. H. Bok

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Résumé de l'article

Le DiPel™, une formulation commerciale du *Bacillus thuringiensis* var. *kurstaki* (*Btk*), et des formulations bio-encapsulées du *Btk* ont été pulvérisées dans un verger de pommiers. Pendant trois années consécutives, leur persistance a été évaluée en laboratoire sur des larves de tordeuse à bandes obliques (*Choristoneura rosaceana*). En 1995, le DiPel et la formulation bio-encapsulée ont causé une mortalité larvaire significativement plus grande que celle du témoin, au moins 1 jour et jusqu'à 27 jours après les traitements, respectivement. En 1996, bien que 6 mm de pluie soient tombés la journée des traitements, le DiPel et la formulation bio-encapsulée ont causé respectivement 21 et 33 % de mortalité larvaire immédiatement après les traitements. Le DiPel a causé une mortalité significativement plus grande que celle du témoin pour une période de trois jours après les traitements alors que la formulation bio-encapsulée a causé une mortalité plus grande pendant 14 jours. En 1997, deux souches de *Btk* (HD-1 et BMP123) ont été pulvérisées à des concentrations différentes en matière active (3 et 8 %) et en doses (30 et 240 g 15 L<sup>-1</sup>). Des concentrations élevées en matière active et des doses élevées ont accru la persistance de l'activité insecticide des formulations de *Btk*. Parmi les facteurs météorologiques mesurés (précipitations, température et radiations solaires), les précipitations ont réduit la persistance de l'activité insecticide. Les radiations solaires peuvent également avoir contribué à moduler la persistance des formulations.

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## **Persistence of insecticidal activity of novel bio-encapsulated formulations of *Bacillus thuringiensis* var. *kurstaki* against *Choristoneura rosaceana* [Lepidoptera: Tortricidae]**

Jean-Charles Côté<sup>1</sup>, Charles Vincent<sup>1</sup>, Kwang-Hee Son<sup>2</sup>, and Song Hae Bok<sup>2</sup>

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DiPel™, a registered *Bacillus thuringiensis* var. *kurstaki* (*Btk*)-based formulation, and experimental bio-encapsulated *Btk* formulations were sprayed in an apple orchard. Their persistence was assessed in the laboratory against obliquebanded leafroller (*Choristoneura rosaceana*) larvae for three consecutive years. In 1995, DiPel and the bio-encapsulated formulation caused significantly higher larval mortality than the control at least 1 day, and up to 27 days after treatments, respectively. In 1996, although 6.4 mm of rain fell during the day of treatments, DiPel and the bio-encapsulated formulation caused respectively 21 and 33% larval mortality immediately after treatments. DiPel caused significantly higher mortality than the control for up to 3 days while the bio-encapsulated formulation caused significantly higher mortality for up to 14 days. In 1997, two *Btk* strains (HD-1 and BMP123) were sprayed at different concentrations in a.i. (3 and 8%) and doses (30 and 240 g 15 L<sup>-1</sup> applied). Higher concentrations of a.i. in the *Btk* formulations and higher doses sprayed increased the persistence of the insecticidal activity. Among the meteorological factors that were measured (i.e. precipitations, temperature and solar radiations), precipitations reduced the persistence of the insecticidal activity. Solar radiations may have modulated the persistence of the formulations.

**[Persistance de l'activité insecticide de formulations bio-encapsulées du *Bacillus thuringiensis* var. *kurstaki* contre *Choristoneura rosaceana* [Lepidoptera : Tortricidae]]**

Le DiPel™, une formulation commerciale du *Bacillus thuringiensis* var. *kurstaki* (*Btk*), et des formulations bio-encapsulées du *Btk* ont été pulvérisées dans un verger de pommiers. Pendant trois années consécutives, leur persistance a été évaluée en laboratoire sur des larves de tordeuse à bandes obliques (*Choristoneura rosaceana*). En 1995, le DiPel et la formulation bio-encapsulée ont causé une mortalité larvaire significativement plus grande que celle du témoin, au moins 1 jour et jusqu'à 27 jours après les traitements, respectivement. En 1996, bien que 6 mm de pluie soient

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1. Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, 430 Gouin Blvd, Saint-Jean-sur-Richelieu (Quebec), Canada J3B 3E6; e-mail: cotejc@em.agr.ca
  2. Korea Research Institute in Biosciences and Biotechnology, P.O. Box 115, Yu-Sung, Taejeon 305-600, Korea

tombés la journée des traitements, le DiPel et la formulation bio-encapsulée ont causé respectivement 21 et 33 % de mortalité larvaire immédiatement après les traitements. Le DiPel a causé une mortalité significativement plus grande que celle du témoin pour une période de trois jours après les traitements alors que la formulation bio-encapsulée a causé une mortalité plus grande pendant 14 jours. En 1997, deux souches de *Btk* (HD-1 et BMP123) ont été pulvérisées à des concentrations différentes en matière active (3 et 8 %) et en doses (30 et 240 g 15 L<sup>-1</sup>). Des concentrations élevées en matière active et des doses élevées ont accru la persistance de l'activité insecticide des formulations de *Btk*. Parmi les facteurs météorologiques mesurés (précipitations, température et radiations solaires), les précipitations ont réduit la persistance de l'activité insecticide. Les radiations solaires peuvent également avoir contribué à moduler la persistance des formulations.

## INTRODUCTION

Formulation is a key component for the success of microbial pesticides. As defined by Burges and Jones (1998), a microbial pesticide «formulation comprises aids to preserving organisms, to delivering them to their targets, and once there, to improve their activities».

Several *Bacillus thuringiensis* var. *kurstaki* (*Btk*)-based commercial formulations are used as alternatives to chemical insecticides for the control of lepidopteran pests. They suffer, however, from short persistence caused by rain washing, lack of adherence on leaf surface and degradation of biologically active proteins by UV light (Frye *et al.* 1973; Pinnock *et al.* 1971; van Frankenhuyzen and Nystrom 1989). In field situations, sunlight, especially wavelengths ca. 300 nm, is most destructive to *Bacillus thuringiensis* spores and protoxins (Jones and Burges 1998). Consequently, several applications must be done each season to achieve adequate crop protection. This limits the usefulness of *Btk* formulations that are commercially available.

To address the problem of short persistence, longer lasting *Btk* formulations have been developed. Encapsulation technology, a process whereby a chemical pesticide is coated with synthetic polymers, has been used initially to protect chemical pesticides from degradation in the environment or, alternatively, to provide controlled release

of the active ingredient (Raun and Jackson 1966). Similar technologies have been developed for microbial pesticides. *Btk* has been encapsulated with clay (Ahmed *et al.* 1973; Raun and Jackson 1966), UV-absorbing compounds (Hostetter *et al.* 1975), chemical polymer matrices such as polyvinylpyrrolidone and polyvinylalcohol (Baker *et al.* 1987), and in a starch matrix without the use of chemical cross-linking agents (Dunkle and Shasha 1988, 1989). The latter, referred to as bio-encapsulation, uses an abundant source of natural biopolymer as coating materials. It uses mild conditions during the bio-encapsulation process so that up to 70% of the insecticidal activity is retained after a 2-wk period in simulated rain in a greenhouse (McGuire and Shasha 1990).

The bio-encapsulation process of *Btk* and other microorganisms has been further modified by Bok *et al.* (1993). This chemical-free technique uses a series of inexpensive biopolymers such as potato starch combined with rice flour and rye, barley and soybean powders. These polymers act as stickers to provide improved adhesiveness to the plant leaves. By entrapping the active ingredient, they could presumably retain its entomocidal activity through better protection against dessication, sunlight, heat and the damaging effects of UV-light (Bok *et al.* 1993).

The obliquebanded leafroller, *Choristoneura rosaceana* Harris [Lepidoptera : Tortricidae], is a polyphagous insect that

became resistant to several synthetic insecticides in some apple growing areas of North America, including New York state (Reissig 1978; Reissig *et al.* 1986) and Quebec (Carrière *et al.* 1994; Smirle *et al.* 1998). Alternatives to synthetic insecticides with different modes of action are needed to alleviate or delay resistance. *Btk* formulations would be particularly useful in this context. In Nova Scotia apple orchards, Hardman and Gaul (1990) found that obliquebanded leafroller fruit injury was 0.25% following DiPel™ application (3.48% fruit injury in the control, with no significant difference). Commercial formulations of *Btk* have been assayed in raspberry fields. The half-life of DiPel WP and Foray 48B ranged from 2.45 to 6.68 d, depending on application rate or spray volume (Li and Fitzpatrick 1996). Higher residual activity was obtained at higher application rates and at lower spray volumes. *Btk* formulations should ensure adequate larval control, provided that the formulations would remain active for a sustained period on the foliage.

Little has been published on the persistence of long lasting formulations in field situations, most notably in agricultural tree-fruit situations. In the present study, bio-encapsulated formulations of *Btk* were assessed for their residual activity in an apple orchard located in Frelighsburg (Quebec) in summers 1995, 1996 and 1997, using bio-assays against obliquebanded leafroller larvae.

## MATERIALS AND METHODS

### Bacterial strains

*Btk* strain HD-1 was isolated from DiPel, a formulation developed by Abbott Laboratories, Chemicals and Agricultural Products Division, North Chicago, Illinois, U.S.A. *Btk* strain BMP-123 was obtained from T. Couch, Becker Microbials, Plantation, Florida, U.S.A.

### Bio-encapsulation of *Bacillus thuringiensis*

The bio-encapsulated *B. thuringiensis* was prepared as described in Bok *et al.* (1993). In summary, a culture of *Btk* strain HD-1 or strain BMP-123 was

grown in SYG medium (2% soytone, 1% glucose, 0.2% yeast extract, 0.1%  $K_2HPO_4$ , 0.1%  $KH_2PO_4$ , 0.05%  $MgSO_4 \cdot 7H_2O$ , 0.02%  $MnCl_2$ , 0.02%  $ZnSO_4 \cdot 7H_2O$ , 0.02%  $FeSO_4 \cdot 7H_2O$ , pH 7.0) at 30°C, for 5 d until sporulation. The sporulated culture was centrifuged at 10 000 X g for 30 min at 4°C. A biogel matrix was prepared by mixing the following series of powders and salts (100 g rice powder, 100 g soybean powder, 10 g cotton seed powder, 2 g glucose, 1 g  $CaCO_3$ , 1 g yeast extract, 20 g soil, 50 mg  $FeSO_4 \cdot 7H_2O$  and 10 mg  $MnCl_2 \cdot 4H_2O$ ). The mixture was boiled for 1 h with vigorous stirring and autoclaved at 121°C for 30 min. After cooling,  $2 \times 10^{11}$  spores were added and thoroughly mixed. The gel was spread on a plate and dried at room temperature for 2 d. The dried complex was ground to a fine powder of 75-100  $\mu m$  in diam containing  $5 \times 10^8$  spores  $g^{-1}$ .

### *Bacillus thuringiensis* formulations and evaluation of B.I.U.

DiPel WP, a *Btk* strain HD-1-based wettable powder, was used as a standard. The commercial label indicated that the formulation contained 16 billion International Units (B.I.U.) per kg. The number of B.I.U. in DiPel and in the bio-encapsulated formulations of *Btk* HD-1 and *Btk* BMP123 were determined by standardized bioassays on *Trichoplusia ni* (Hbn.) [Lepidoptera : Noctuidae] larvae as described by Dulmage *et al.* (1971). In 1995, laboratory bioassays on *T. ni* determined that the bio-encapsulated product had, on an equal weight basis, ca. 12.5% the number of B.I.U. contained in DiPel. Instead of equal amount of formulations we decided to spray equivalent amount of a.i., as measured in B.I.U., of DiPel and bio-encapsulated *B. thuringiensis*. Consequently, on a weight to weight basis, eight times more bio-encapsulated *B. thuringiensis* were sprayed in the field in 1995 compared to DiPel, so that equivalent number of B.I.U. per applied dose were used (Table 1).

In 1996, laboratory bioassays determined that the bio-encapsulated Bt formulation contained 10% the number of B.I.U. of DiPel on an equal weight basis.

Consequently, on a weight to weight basis, 10 times more bio-encapsulated *Bacillus thuringiensis* product than DiPel were sprayed to ensure that equivalent amount of biologically-active material were used (Table 1).

In 1997, laboratory bio-assays determined that, on an equal weight basis, the bio-encapsulated Bt formulations H3 and B3 contained 12.5% the amount of B.I.U. of DiPel, and formulations H8 and B8, 33% (Table 1).

### Field trials

All trials were done at the Agriculture and Agri-Food Canada Experimental Farm located at Frelighsburg (45°03' N, 75°50' W), Quebec, in an apple orchard composed of cultivar "Liberty". The field layout consisted of several experimental units, each comprising five contiguous trees in a row per treatment, with a row of trees acting as buffer between each treated rows. The field layouts were similar in 1995, 1996 and 1997. The treatments were applied directly on apple trees with a handgun until

runoff on 16 August 1995, 9 July 1996 and 4 September 1997.

Meteorological data were collected by an automatic weather station located on the farm ca. 600 m from the plots. Solar radiation ( $\text{MJ m}^{-2}$ ) was measured with a pyranometer (LI-200SZ) (Li-Cor, Lincoln, Nebraska, U.S.A.) that had a sensor with a defined response from 385 to 1110 nm.

### Laboratory bioassays of Btk persistence in the field

To assess the residual activity of the formulations, 15 apple leaves were randomly collected on trees of each treatment at different dates after treatment (see Tables 2, 3 and 4 for collecting schedule). They were immediately brought to the laboratory in a refrigerator where they were kept for a few hours until the bioassays. Leaf disks of 15 mm diam were cut with a metal auger. Third instar larvae were individually starved for 24 h before the bioassays and individually offered one leaf disk (treated or control) for 24 h. The

**Table 1. Summary of dates of treatments, treatments applied, a.i. used, number of billion International units (B.I.U.)  $\text{kg}^{-1}$ , applied dose ( $\text{g 15 L}^{-1}$ ) and B.I.U. per applied dose**

Dates of treatments (no. of larvae tested per treatment)	Treatments <sup>a,b</sup>	Active ingredients	B.I.U. $\text{kg}^{-1}$	Applied dose ( $\text{g 15 L}^{-1}$ applied)	B.I.U./ applied dose
16 August 1995 (n = 30)	Control	Water			
	DiPel WP	HD-1 DiPel	16	30	0.48
	H3-240	HD-1	2	240	0.48
9 July 1996 (n = 40)	Control	Water			
	DiPel WP	HD-1 DiPel	16	30	0.48
	H2-300	HD-1	1.6	300	0.48
4 September 1997 (n = 40)	Control	Water			
	DiPel WP	HD-1 DiPel	16	30	0.48
	H3-30	HD-1	2	30	0.06
	H8-30	HD-1	5.3	30	0.16
	B3-30	BMP123	2	30	0.06
	B8-30	BMP123	5.3	30	0.16
	H3-240	HD-1	2	240	0.48
	B3-240	BMP123	2	240	0.48
	DiPel WP	HD-1 DiPel	16	240	3.84
	H8-240	HD-1	5.3	240	1.28
	B8-240	BMP123	5.3	240	1.28

<sup>a</sup> Tween®20 was added at a 0.1% level to each treatment, including the controls.

<sup>b</sup> Acronym for the bio-encapsulated formulation : the first letter, H or B, stands for *Bacillus thuringiensis* var. *kurstaki* strains HD-1 and BMP123, respectively; the first number, 2, 3 or 8, stands for the percent of *Bacillus thuringiensis* a.i. in the formulation; the second number, 30, 240 or 300, stands for the amount (g) of bio-encapsulated products suspended in 15 L of water and applied to trees.

**Table 2. Percent larval mortality of obliquebanded leafroller six days following ingestion of *Btk* treated leaf disks taken from leaves collected on apple trees sprayed on 16 August 1995<sup>a</sup>**

Days after treatment	Larval mortality (%) <sup>b</sup>		
	Control	DiPel	H3-240
0	7	87*	72*
1	7	47*	67*
6	7	10	53*
13	0	0	30*
20	7	11	30*
27	3	7	14*

<sup>a</sup> see Table 1 for description of treatments.<sup>b</sup> n = 30 larvae per treatment.\* significantly different from the control ( $P = 0.05$ , test for comparisons of proportions, Zar 1996).**Table 3. Percent larval mortality of obliquebanded leafroller six days following ingestion of *Btk* treated leaf disks taken from leaves collected on apple trees sprayed on 9 July 1996<sup>a</sup>**

Days after treatment	Larval mortality (%) <sup>b</sup>		
	Control	DiPel	H3-240
0	0	21*	33*
1	10	5	18
3	0	8*	15*
7	3	3	10
10	0	0	8*
14	0	3	8*

<sup>a</sup> see Table 1 for description of treatments.<sup>b</sup> n = 40 larvae per treatment.\* significantly different from the control ( $P = 0.05$ , test for comparisons of proportions, Zar 1996).**Table 4. Percent larval mortality of obliquebanded leafroller six days following ingestion of *Btk* treated leaf disks taken from leaves collected on apple trees sprayed on 4 September 1997<sup>a</sup>**

Days after treatment	Larval mortality (%) <sup>b</sup>										
	Group A <sup>c</sup>				Group B			Group C			
	Control	DiPel 30 <sup>d</sup>	H3-30	H8-30	B3-30	B8-30	H3-240	B3-240	DiPel 240	H8-240	B8-240
0	2.6	25.6 a	12.8 a,b	5.1 b	5 b	15.8 a	33.3 a	20 a	67.5 a,b	46.2 a,b,c	53.8 a,b
1	0	5	2.5	2.5	0	0	17.5 a,b	15.8 a,b	71.8 a,b	35.9 a,b,c	25 a,b,c
5	0	7.5 a	0 b	0 b	2.6	0 b	10 a	7.5 a	39.5 a,b	20 a,b,c	20 a,b,c
12	0	2.5	10 a	2.5	0	5.3	0	0	15.4 a,b	5 c	12.5 a,b
19	0	2.5	0	2.5	5	5.1	0	5	5	0	0
26	2.5	5	2.6	0	0	0	5	2.5	0	2.5	10

<sup>a</sup> see Table 1 for description of treatments.<sup>b</sup> n = 40 larvae per treatment.<sup>c</sup> Group A: treatments applied on trees at 30g L<sup>-1</sup> with bio-encapsulated formulations containing 3 or 8% a.i.; group B: treatments applied on trees at 240g L<sup>-1</sup> with bio-encapsulated formulations containing 3% a.i.; group C: treatments (i.e. the commercial standard DiPel or bio-encapsulated formulations containing 8% a.i.) applied on trees at 240g L<sup>-1</sup>.<sup>d</sup> Treatment comparisons and significance ( $P = 0.05$ , test for comparisons of proportions, Zar 1996): percent followed by an "a" are significantly different from the control; within group A, B, and C, percent followed by a "b" are significantly different from that of DiPel-30; and within group C, percent followed by a "c" are significantly different from DiPel-240.

larvae were then transferred individually into a plastic Solo® cup (Solo Cup Co., Urbana, Illinois, U.S.A.) containing a pinto bean-based artificial diet (modified from Shorey and Hale 1965). The cups were kept at 24°C and 16L:8D photoperiod. Larval mortality was recorded daily for 6 d.

### Statistical analysis

For each date after the treatment, statistical differences between larval mortality in treatments and the control were detected by using the test for comparing proportions with  $P = 0.05$  (Zar 1996, box 23.23). In 1995 and 1996, percent of larval mortality in the treatments was

compared to that of the control. In 1997, recorded percent of larval mortality were compared respectively with 1) the control, 2) the commercial standard at the recommended dose (i.e. DiPel 30) and 3) the commercial standard at a high dose (i.e. DiPel 240).

## RESULTS

In preliminary trials and bioassays conducted in July 1995, larval mortality caused by DiPel immediately after treatment was five-fold higher than mortality caused by the bio-encapsulated *B. thuringiensis* when equal amount of formulated products were used (data not shown). Because of the large total amount of ingredients used for bio-encapsulation, the relative amount of *B. thuringiensis*, the a.i. measured in B.I.U.  $\text{kg}^{-1}$ , was lower in the bio-encapsulated product than in DiPel (Table 1). A second series of treatments was conducted on 16 August 1995 with equivalent amount of B.I.U. per applied dose. Larval mortality in the control varied from 0 to 7% (Table 2). Immediately after treatment, DiPel caused 87% larval mortality. Twenty-four h later, larval mortality was down to 47% and subsequent mortality was not significantly different from that of the control, ranging from 0-11%. The bio-encapsulated *B. thuringiensis* exerted control comparable to DiPel for the first 24 h. Killing efficiency was, however, significantly different from the control for d 6, 13, 20 and 27 after treatment. Maximum temperatures for the period comprised between 16 August and 12 September 1995 slowly decreased from ca. 30 to 15°C (Table 5). Following spraying, slight precipitations of 5, 5.4 and 11 mm of rain were recorded on d 13, 15 and 22, respectively. The cumulative solar radiation at d 1, 6, 13, 20 and 27 after treatment was 47.2, 156.9, 278.5, 402.7 and 516.2  $\text{MJ m}^{-2}$ , respectively.

In 1996, larval mortality in the control ranged from 0-10% throughout the course of the experiment (Table 3). DiPel caused a significantly higher larval mortality than the control immediately and 3 d after treatment. The bio-encap-

sulated *Btk* formulation H2-300 gave a significantly higher percent of larval mortality than the control at 0, 3, 10 and 14 d after treatment. Maximum temperatures between 9 July and 30 July 1996 ranged in the mid '20s (Table 5). Several d with precipitation were recorded during the period under study, i.e. 6.4, 8, 3.2, 26, 24, 22.8 and 17.8 mm of rain registered 0, 4, 5, 6, 7, 10 and 11 d respectively following spraying. The cumulative solar radiation at d 1, 3, 7, 10 and 14 after treatment was 37.7, 87.2, 133.8, 185.1 and 259.2  $\text{MJ m}^{-2}$ , respectively.

In 1997, a more thorough trial was conducted. A first series of treatments was done on 12 August (data not shown). The data were discarded because heavy rain fell after the treatment. A second treatment was applied on 4 September 1997 (Table 4). Two *Btk* strains were sprayed, HD-1 and BMP123. Both strains were bio-encapsulated at different concentrations of B.I.U.  $\text{kg}^{-1}$  and applied in the field at different doses (Table 1). Hence, samples with equivalent amount of a.i. but different amount of final formulations, as well as samples with equal amount of total material but different amount of a.i., were sprayed to study the effect on larval mortality of 1) the concentration in a.i., and 2) the amount of total product used. Low ( $\leq 2.6\%$ ) larval mortality was recorded in the control throughout the experiment (Table 4). Mortality caused by DiPel 30 was 25.6% after treatment and subsequently  $\leq 7.5\%$ . DiPel 240 caused ca. 70% mortality the first 24 h following treatment, and later mortality was significantly different from that of the control up to 12 d after treatment. All bio-encapsulated *Btk* formulations of group A (i.e. H3-30, B3-30, H8-30 and B8-30) caused  $\leq 16\%$  larval mortality immediately after treatment. Later mortality was not significantly different from that of the control, except for H3-30 at d 12 where 10% larval mortality was recorded.

Bio-encapsulated *Btk* formulations containing 3 or 8% a.i., applied at a rate of 240 g 15  $\text{L}^{-1}$  of water, (i.e. H3-240 and B3-240 in Group B; H8-240 and B8-240 in Group C) caused significantly more

**Table 5. Meteorological conditions that prevailed after treatments of apple trees with *Btk* formulations, in Frelighsburg, Quebec, Canada. Dates of treatments appear between parentheses beside the year**

Day after treatments	1995 (16 August)				1996 (9 July)				1997 (4 September)			
	Max. (°C)	Min. (°C)	Precipitations (mm)	Solar radiation (MJ m <sup>-2</sup> )	Max. (°C)	Min. (°C)	Precipitations (mm)	Solar radiation (MJ m <sup>-2</sup> )	Max. (°C)	Min. (°C)	Precipitations (mm)	Solar radiation (MJ m <sup>-2</sup> )
0	28.3	18.0	0	24.5	25.0	13.3	6.4	21.7	14.5	6.7	0	11.0
1	30.2	15.7	0	22.7	19.4	13.8	1	16.0	21.6	8.0	0	20.7
2	26.9	15.6	0	23.7	24.6	12.4	0	27.3	18.9	14.4	0	6.9
3	26.5	14.4	0	24.5	26.5	14.4	0	22.2	17.6	9.6	0	3.3
4	29.4	17.3	0	24.2	21.8	17.4	8	5.7	21.2	5.7	0	18.2
5	29.1	19.1	0	24.2	27.0	16.8	3.2	14.5	23.4	8.3	1.2	17.2
6	19.7	14.5	0	13.1	25.3	19.5	26	7.8	22.6	12.5	0	15.0
7	21.2	11.1	0	16.9	25.5	17.4	24	18.6	22.5	15.9	0	9.7
8	20.7	11.2	0	10.3	25.8	17.3	0	21.0	23.8	14.6	2.8	11.0
9	20.3	9.9	0	16.8	28.0	16.0	0	24.0	21.6	13.5	0	13.4
10	21.2	8.7	0	15.4	24.8	14.9	22.8	6.3	22.8	14.0	1.4	8.9
11	21.7	7.2	0	19.1	15.6	12.1	17.8	5.6	22.3	13.0	0	11.5
12	24.3	4.1	0	24.3	23.0	12.7	1	25.2	17.1	7.8	0	12.3
13	24.7	12.2	5	18.8	26.6	10.6	0	27.0	21.4	5.4	4.6	16.3
14	22.2	8.4	0	23.2	26.9	13.5	3.7	16.3	21.8	12.9	0	15.8
15	21.0	11.9	5.4	7.4	27.9	16.6	1.1	22.3	23.4	11.8	0	11.6
16	20.5	11.8	0	15.0	24.9	19.2	2.2	11.8	17.9	7.4	26.2	1.5
17	20.4	7.7	0	19.9	25.3	16.7	2	18.1	10.4	2.3	1	9.3
18	22.2	5.4	0	20.4	22.2	14.5	0	17.6	17.4	1.7	0	15.2
19	24.7	11.1	0	21.1	23.0	11.2	0	24.1	13.6	1.9	8.6	2.9
20	25.9	11.9	0	17.2	26.3	9.7	0	21.4	9.9	-0.6	0	11.8
21	21.2	7.4	0	23.2	23.4	17.5	0	10.6	16.6	5.0	0	13.1
22	24.9	6.7	11	6.4	- <sup>a</sup>	-	-	-	11.3	7.0	2.8	1.7
23	16.4	2.9	0	22.2	-	-	-	-	16.0	1.2	0	16.0
24	16.4	6.6	2	6.1	-	-	-	-	20.2	7.8	1.2	16.1
25	14.2	3.5	0	16.5	-	-	-	-	14.8	11.1	26.4	2.6
26	19.6	0.7	0	19.2	-	-	-	-	15.5	6.9	5.9	4.6
27	24.1	11.1	0	19.9	-	-	-	-	-	-	-	-

<sup>a</sup> Data not presented because the experiment was terminated.

mortality than the control up to 5 d after treatment except for formulation B8-240, where mortality was significantly greater than the control up to 12 d after treatment.

Mortalities recorded with all bio-encapsulated formulations and with DiPel 240 (Groups A, B and C) were compared to the ones caused by DiPel 30. Bio-encapsulated formulations H3-30 and B3-30, and formulations H8-30 and B8-30 (Group A), contained respectively 1/8 and 1/3 the amount of a.i. found in DiPel 30 (Table 1). All four experimental formulations caused comparable or less larval mortality than DiPel 30. Bio-encapsulated formulations H3-240 and B3-240 (Group B), and formulations H8-240 and B8-240 (Group C), contained

respectively one-fold and 2 2/3-fold the amount of a.i. found in DiPel 30. Bio-encapsulated formulations H3-240 and B3-240 caused significantly more mortality than DiPel 30, 24 h after treatments whereas DiPel 240, H8-240 and B8-240 caused significantly more larval mortality than DiPel 30, at least 5 d after treatment and up to 12 d after treatment for DiPel 240 and B8-240.

Mortality recorded with H8-240 and B8-240 (Group C) was compared to that caused by DiPel 240. Both bio-encapsulated formulations contained 1/3 the amount of a.i. found in 240 g of the commercial formulation, DiPel 240. DiPel 240 caused significantly higher mortalities than both bio-encapsulated formulations up to 5 d after treatment.



Maximum temperatures for the period comprised between 4 and 30 September varied from 9.9 to 23.8°C (Table 5). Minimum temperatures varied from -0.6 to 15.9°C. Two d with major precipitations of 26.2 and 26.4 mm of rain were recorded respectively 16 and 25 d after treatment. The cumulative solar radiation at d 1, 5, 12, 19 and 26 after treatment was 31.7, 77.3, 159.1, 231.7 and 297.6 MJ m<sup>-2</sup>, respectively.

## DISCUSSION

A variety of factors may limit the efficacy of these *B. thuringiensis* var. *kurstaki* formulations, short persistence being a key one. Longer lasting formulations would offer several advantages over currently registered *Btk* formulations. Because of the increased persistence of the activity on the foliage, less spraying would be required, thus reducing costs related to product and labor. The sustained activity of longer lasting *Btk* formulations could allow a greater flexibility in interpreting an optimal window of application. This feature can be useful, as in the case of the obliquebanded leafroller, an insect pest of apple orchards, where oviposition can span up to one month. Although obliquebanded leafroller larvae may be differentially sensitive to *Btk* (Li *et al.* 1995) and although eggs, pupae and adults will usually escape exposure (Rigby 1991), long-lasting residual activity may increase the likelihood that vulnerable stages may ingest the toxins.

Several abiotic factors (e.g. UV light, heat, rain, etc.) may affect the persistence of *Btk* formulations and, in field situations, the effect of these factors may be simultaneous and confounding (Jones and Burges 1998). Our results suggest that precipitation was a key factor in the non-persistence of the insecticidal activity. Under meteorological conditions with low rainfall, as exemplified in summer 1995, the bio-encapsulated *Bacillus thuringiensis* lasted longer in the apple orchard than DiPel. In 1996, however, rain on the d of spraying (Table 5) appears to have reduced the persistence, and hence the activity, of both DiPel and the experi-

mental *Btk* formulations (Table 3). Low mortality was recorded 24 h after spraying. In 1997, no rain fell during the first 4 d after treatment and low precipitation (< 5 mm per d) fell occasionally during the next 11 d.

Solar radiation that prevailed during the course of our field trials varied from yr to yr. For example, in the first 6 d after treatment, 156.9, 115.2 and 92.3 MJ m<sup>-2</sup> were measured in 1995, 1996 and 1997, respectively. Likewise, in the first 14 d after treatments, 301.7, 259.2 and 191.2 MJ m<sup>-2</sup> were measured in 1995, 1996 and 1997, respectively. Solar radiation may have played a role in modulating the persistence of the *Btk* formulations in the field.

In contrast to the precipitation that often was negligible most of the time during the course of the trials but sporadically occurred in large quantities in some d (e.g. d 6, 7, 10 and 11 in 1996), solar radiation accumulated steadily every d. Few days had < 10 MJ m<sup>-2</sup> of radiation, respectively 3, 3 and 10 d in 1995, 1996 and 1997.

In Quebec, meteorological conditions (e.g. rain, temperature and solar radiation) are often unpredictable and very variable throughout the season (G. Bourgeois, personal communication). These constitute very conservative conditions for field trials of pesticide formulations.

Among the manageable factors, concentration of a.i. in the formulation, total amount of formulation sprayed and identity of the bacterial strain used may influence the outcome of the trials but they must comply to registration and economic contingencies.

The concentration of a.i. in the formulations and the amount of total formulated powder sprayed have played key roles in increasing the persistence of the insecticidal activity of the *Btk* formulations, as exemplified in 1997. The greater concentration in a.i. and the greater amount of product used, the more likely the residual insecticidal activity will be retained over a longer period of time, as also concluded by Li and Fitzpatrick (1996).

Field trials do not allow to experimentally single out one factor, but they are conveniently suited to address the agronomic and variable meteorological conditions prevailing in commercial settings. The next logical step would be to perform large scale trials in commercial orchards to generate data such that registration of such formulations could be done.

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